HINDUSTAN ANTIBIOTICS

Bulletin

AUGUST 1960

VOL. 3

1

HINDUSTAN ANTIBIOTICS
Bulletin

Vol. 3 **August 1960** **No. 1**

EDITORIAL & ADVERTISEMENT OFFICES

Hindustan Antibiotics Ltd., Pimpri, Poona Dist.

This *Bulletin* is published on the 15th of February, May, August & November, and contains articles and reviews on all aspects of antibiotics production and use.

The views expressed in this journal are those of the authors and do not necessarily represent those of the Company or of the editors.

Annual subscription : Rs. 3.00 (inland), 8 sh. or $1.50 (foreign). Single copy: Re. 1.00 (inland), 2sh. 6d. or $0.50 (foreign).

CONTENTS

Incentives and Rewards 1

Editorial

Penicillinase in Penicillin Therapy 2

Review

Stainless Steels for Process Equipment .. 3
P. N. Devarajan

Original Papers

Factors Influencing Penicillin Titres in Industrial Fermentors 11
R. S. Gondhalekar, R.S. Phadke

Production of *Penicillium* mutants by Ultrasonic Waves and Shortwave Diathermy 21
K. S. Gopalkrishnan, N. J. Waichal

Control of Root Rots of Crop Plants with Nystatin 24
K. R. Rao, M. J. Thirumalachar

Short Notes

Simple Air-lift Laboratory Bottle Fermentor 28
D. Ghosh

"Maxipen" 30

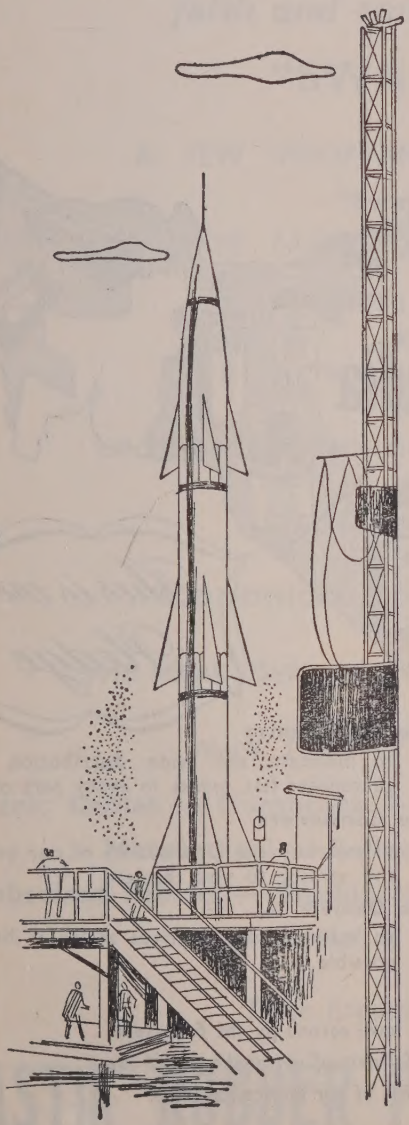
Portrait of a Company

Glaxo Laboratories 31

Advertisers' Index A4

ADVERTISERS INDEX

Alembic Chemical Works Co. Ltd.	A 10
Blue Star Engineering Co.	A 9
Blue Star Engineering Co.	A 17
British Drug Houses (India) (P) Ltd.	A 20
Chika Private Ltd.	A 18
Dumex (P) Ltd.	A 14
East India Pharmaceutical Works Ltd.	A 19
Franco-Indian United Laboratories	A 5
Geoffrey Manners and Co. (P) Ltd.	A 6
Glaxo Laboratories (India) (P) Ltd.	Inside back cover
Imperial Chemical Industries	A 11
Indian Process Chemical Laboratories	A 21
JG Glass Industries	A 12
Kemp and Co. Ltd.	A 13
Lyka Laboratories	A 21
Mitsui Bussan Kaisha Ltd.	A 16
Oriental Pharmaceutical Industries Ltd.	A 4
Parry and Co. Ltd.	Outside back cover
Sarabhai Chemicals	A 8
Swastik Oil Mills	A 15
Swastik Rubber Products Ltd.	A 7
Union Carbide India Ltd.	Inside front cover



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INCENTIVES & REWARDS

"IN an expanding economy the rich become richer and the poor become poorer and there is very little that a democratic Government can do to prevent it. An inevitable result of such concentration of economic power is the corruption of the political power leading to a totalitarian government. Industries in the public sector by preventing too much concentration of economic power, serve as a check on this tendency and help the people in having a stable democratic government. The first reward for the officer of the public sector is, thus, his significant role in nation building. Further, the private sector can afford to pay very well really good men, but these talented men have to help in the exploitation of the masses. The second reward for an officer of the public sector is that he is not being a party to this exploitation.

As to the question of monetary incentives and rewards it will have to be appreciated that in any big venture many people try more or less equally hard and conscientiously but only few are lucky to succeed. If rewards were offered to these it may, obviously, pep them up, but it may also create frustration in the minds of those who did not succeed in spite of equally hard work. It may even lead some persons to suspect favouritism or nepotism. The nationalised industry is by the very nature of things, an impersonal body where monetary rewards have to be rather limited. It should not, however, prevent a good man from doing his best as long as he is getting basically a satisfactory wage."

Extract from a valedictory address given by S. T. Raja, Managing Director, Hindustan Antibiotics Ltd., to the 7th Session of the Field Officers' Training Unit of Life Insurance Corporation, at Poona.

Penicillinase in Penicillin Therapy

PENICILLIN, the first therapeutically successful antibiotic, is still the most important one in use. In United States of America alone, over 500 tons are manufactured every year, and other countries including India are fast increasing their penicillin production. Abraham and Chain in 1940 discovered a penicillin destroying enzyme in *Escherichia coli* a gram-negative bacteria and named it penicillinase. Several bacteria have since been shown to produce penicillinase and this inactivation has hitherto been regarded as an obstacle in therapeutics. The resistant staphylococci produce penicillinase which enables them to inactivate the antibiotic in their micro-environment and thus continue to multiply.

In order to overcome the neutralizing effects of penicillinase secreted by resistant staphylococci, investigation on new lines of attack against the resistant strains were taken up. Antibody against penicillinase in the form of anti-penicillinase serum was produced in animals. Tacking (1955) and later Markov (1956) successfully immunised guinea pigs with penicillinase or injected the immune serum so produced, to neutralise the penicillinase produced by the resistant staphylococci at the site of infection. In such cases penicillin even at low concentrations completely inhibited the resistant staphylococci. While this antipenicillinase serum-antibiotic therapy has been very successfully employed in animals, it has not been exploited in man.

As already stated, penicillinase is produced by a large number of bacteria, among which *Bacillus cereus* and *Staphylococcus aureus* are important. Recent studies have shown that the penicillinase produced by *B. cereus* and *S. aureus* may have immunological differences. Till now, penicillinase has been used chiefly in the sterility tests of the antibiotic itself. The enzyme was at first considered to be intracellular, but now it has been shown to be in cell-free state in broth cultures. It can be adsorbed on Seitz, Berkfeld and glass filters. Commercially it has been produced from *B. cereus* in a pure state by Schenley Laboratories, U.S.A., under the trade name "Neutrapen."

Penicillinase can be useful when destruction of penicillin is needed, and particularly in cases where there are indications of reactions to penicillin therapy. R. N. Becker of U.S.A., was the first to conceive this idea and tested them in animals. Using penicillinase preparation from *B. cereus* he found that the enzyme inactivated penicillin within an hour. The enzyme was well absorbed and circulated in the body, and remained for 4 to 7 days. First clinical trials with penicillinase were reported in 1957 by Minno and Davis in U.S. Navy. Thirty-two cases of penicillin reactions, chiefly in the form of urticaria and pruritus were treated by injecting penicillinase.

As at present known, penicillinase cannot act quickly enough to exert any immediate effect on penicillin shock. The use of anti-histamines, ACTH, and other drugs used at present are still the only means of combating anaphylactic reactions. For other manifestations of sensitivity, which appear long after administration of penicillin, penicillinase may prove to be of considerable value. It is particularly useful in treatment with long acting types of penicillins, where the absorption is prolonged for days, and the reactions have already manifested themselves, with further chances of increase also.

As regards parenteral use of penicillinase preparations, Becker (1956), Zimmerman (1958) and others have shown that it is safe and relatively nontoxic. However, Reisch (1959), Hyman (1959) and others have reported severe reactions in man. As already pointed out, penicillinase may be of value where the penicillin reactions are mild and prolonged.

REVIEW

Stainless Steels for Process Equipment

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SELECTION of materials for process plant and equipment construction is an important function of the design engineer. Prerequisite to success in material selection is an understanding of such factors as structural strength of materials, their resistance to physical and thermal shock, cost, fabrication procedures, maintenance problems, and service expected of the equipment under the operating temperatures, pressures and environmental conditions. These aspects are best brought to light through the joint efforts and co-operation of the designer, fabricator and process plant operator.

Materials of construction may be divided into metals and non-metals. Amongst the various common metallic constructional materials given in Table I, the quantum of differential in initial investment costs is brought out comparing stainless steel material with others.

TABLE I

COMPARISON OF PURCHASED COST OF METAL PLATES

Material	Ratio of $\frac{\text{cost of metal}}{\text{cost of steel}}$
Flange quality steel	1
304 s.s. clad steel	5
316 s.s. clad steel	6
Aluminium	6
304 st. steel	7
Copper	7
Nickel clad steel	8
Monel clad steel	8
Inconel clad steel	9
316 stainless steel	10
Monel	10
Nickel	12
Inconel	13
Hastelloy "C"	40

Stainless steels cost six to ten times as much as carbon steel and have become the quality product of the steel industry. Stainless steel meets the severe demands of modern processing industry at a reasonable cost. It lends itself to fabrication more intricately or more severely at less added cost than other metals by a wide variety of standard factory processes. Corrosion resistance is achieved by proper choice of the material and design of the unit for easy cleaning, the latter also helping to minimize product contamination. Stainless steels received from the mill can be given different textures and degrees of surface finishes, from "dull" to "mirror polish", to suit requirements. The finish can be maintained over long periods with minimum maintenance. Stainless steels do not affect odour, taste and colour of materials handled ; they provide excellent sanitation, and are not attacked by most sterilizing agents. Stainless steels have good strength at both elevated and low temperatures (austenitic type), plus improved resistance to scaling and impact toughness. These properties make them eminently suitable as materials of construction in the pharmaceutical industry. Many "sandwich" combinations of stainless steel and other metals are bonded to form composite clad sheets, which are cheaper than the solid alloys while still offering the advantages of corrosion resistance, freedom from contamination, strength, ductility, etc.

Sixteen of the thirty-five standard wrought stainless steels and five of the eight standard cast stainless steels are recognized by the A.S.M.E. Boiler and Pressure Vessel Code, for use in pressure vessels. The s.s. clad

steels have shear strength at the interface or bond line of the metals over 20,000 p.s.i. which is the minimum specified by the A.S.T.M.

The various aspects of stainless steels, including their metallurgical and chemical composition, suceptibility to and form of corrosion, fabrication procedures, recent developments, etc., are briefly discussed below. A knowledge of these factors would aid the designer, fabricator and product-user to visualize the range, suitability, versatility, as well as the inherent limitations, of stainless steels.

Applications

Stainless steels find extensive use in fabrication of process equipment as well as transfer piping, valves and pumps in the chemical and pharmaceutical industry. In antibiotic plant construction, stainless steels have a very important role because of their corrosion resistance, non-contaminating surface, sterilizable nature and the polish which can be taken and maintained. Here they are used extensively in the fabrication of large fermentors, seed tanks, external heaters and coolers for media sterilization, extraction equipment, crystallizers, evaporators, sterile processing units, milling machines and process piping, valves and transfer pumps. Powder metallurgical techniques have been applied to stainless steel to produce precise parts, lubricant impregnated bearings and porous filters. Such sintered "metafilters" used in the sterile handling of antibiotics have the advantage of being sterilizable, corrosion resistant and of closely controlled porosity and density.

Types of Stainless Steels

Stainless steels can be divided, according to metallurgical structures, into four main groups characterized by their anticorrosive properties :

1. Ferritic steels
2. Martensitic steels
3. Austenitic steels
4. Ferritic-austenitic steels.

Generally, resistance to chemical attack is lower and poorer with the ferritic and martensitic steels as compared to the other types. As the carbon content is increased the strength of 18-8 steels increases but with increased suceptibility to intergranular attack. Manganese and silicon are present as these are used for deoxidizing in the manufacture of special steels. The presence of excess silicon reduces resistance to boiling nitric acid.

Ferritic Steels : Of the various types available, type 405 and 430 are recognized by the A.S.M.E. Boiler and Pressure Vessel Code. These are non-hardenable by thermal treatment. They are fairly ductile and can be fabricated and welded. Cold working reduces their ductility with only slight increase of strength. Ferritic steels are ferromagnetic and resistant to oxidation and corrosion. Stress relieving has to be done by annealing. Type 430 is as good as type 302 (austenitic 18-8 steel) as regards corrosion resistance. The chemical analysis of type 405 and 430 are given in Table II. Ferritic steels find applications in household utensils, cutlery, architectural trimming, general machine construction parts, etc.

TABLE II. FERRITIC STEELS 405 AND 430

AISI type	Percentage by weight					
	Cr	C max.	Mn max.	P max.	S max.	Si max.
405	11.5 / 14.5	0.08	1.0	0.04	0.03	0.5
430	14.0 / 18.0	0.12	1.0	0.04	0.03	1.0

Martensitic Steels : These have carbon-content so proportioned to chromium content (Table III) that on heating above the critical point the steel changes to austenitic structure, while reverting back to martensitic structure on cooling. They are ferromagnetic, hardenable and exhibit best mechanical properties and maximum

TABLE III. MARTENSITIC STEELS

AISI type	Cr	C max.	Al	Mo max.	Zr max.	Se max.	Mn max.	P max.	S max.	Si max.
410 ..	11.5/13.5	0.15	0.10/0.30				1.0	0.04	0.03	1.0
416 ..	12/14.0	0.15		0.6	0.6		1.25	0.06	0.15	1.0
416Se ..	12/14.0	0.15				0.15	1.25	0.06	0.06	1.0

corrosion resistance in hardened and tempered condition. Type 410 is the basic type of the group with 416 and 416Se being free-machining modifications. The martensitic steels are employed for a variety of mild corrosive conditions, in aircraft construction, rock drill tubes, petrol and oil pipes, vacuum brake liners, etc. They resist scaling up to 700°C and are used in constructions involving operating temperatures of 700°C.

Austenitic Steels : These have, in addition to high chromium content of 12-26 per cent, a high nickel content of 7-25 per cent. The nickel is so proportioned to the chromium that the structure becomes austenitic, and the austenitic steels are non-magnetic in the annealed condition. As a group, these possess the high corrosion resistance characteristic of the stainless steels as well as high-temperature resistance. The austenitic stainless steels are inherently tough and strong, but it is their excellent ductility and weldability which distinguish them from other types. The annealed properties of this group show a wide difference between yield point (35,000 p.s.i.) and ultimate strength (90,000 p.s.i). Throughout this "plastic deformation" range, the 300 series (austenitic) have superlative "forming" properties. They are, therefore, well suited for deep drawing and other severe "forming" operations.

Type 304 is the basic austenitic type, others being modifications developed to suit needs. Types 303 and 303Se are free-machining austenitic steels, specified for bolts. Type 309 and 310 are high alloy austenitic steels *viz.* (25-12) and (25-20) ; these have high scaling resistance and are useful in applications of cyclic heating and cooling.

The high-carbon type austenitic stainless steels, without stabilization are subject to chromium carbide precipitation along grain boundaries when passing through the critical temperature range of 800°F to 1600°F. This is likely to occur during cooling after fusion welding, and leads to reduction in corrosion resistance of stainless steel. Low-carbon austenitic stainless steels have been developed to maintain corrosion resistance, since carbon content is of prime importance in the carbide precipitation phenomenon; also temperature and time at temperature are important. Types 304L, 316L and low-carbon 317 types are admirably adapted for use in structures and vessels without annealing after welding. For more critical application, stabilized types 316, 321 and 347 are available. The stabilizers are titanium or columbium-tantalum. These are competitive carbide formers to chromium and thus keep the chromium in the grain boundaries intact. Titanium is added to low-carbon austenetic steels to an extent of 5 times the carbon content, while columbium is added to an extent of 8-10 times carbon content. In types 316 and 317, the low carbon content, coupled with the presence of molybdenum (2-3%), increases corrosion resistance and also confers high temperature strength. The common types in the austenitic group are listed in Table IV. All these types contain 2% Mn, 0.045% P, 0.030% S and 1% Si except type 310 which contains 1.5% of Si.

TABLE IV. AUSTENITIC STAINLESS STEELS

AISI Type	Cr	Ni	C max.	Mo	
302 ..	17.0/19.0	8.0/10.0	0.15		
304 ..	18.0/20.0	8.0/12.0	0.08		
304L ..	18.0/20.0	8.0/12.0	0.03		
309 ..	22.0/24.0	12.0/15.0	0.20		
310 ..	24.0/26.0	19.0/22.0	0.25		
316 ..	16.0/18.0	10.0/14.0	0.08	2.0/3.0	
316L ..	16.0/18.0	10.0/14.0	0.03	2.0/3.0	
317 ..	18.0/20.0	11.0/15.0	0.08	3.0/4.0	
321 ..	17.0/19.0	9.0/12.0	0.08		5 x C of Ti (min.)
347 ..	17.0/19.0	9.0/13.0	0.08		10 x C of Cb-Ta (min.)

Ferritic-Austenitic Steels : These types contain 4-6 per cent of nickel with chromium content of 26 per cent and possess both austenitic and ferritic matrix. Their carbon content is low, and they are non-hardenable.

Corrosive attack by sulphuric acid at various concentrations and temperatures posed a problem to metallurgists and process chemists. From the general range of austenitic steels, Duriron and Co., developed an alloy in cast form called Durimet 20 with the following analysis : Ni 29, Cr 20, Mo 2, Cu 4, C 0.07, Si 1.0, and Fe making up the balance. Alloy-20 was adapted to wrought form by the introduction of rare earths like cerium and lanthanum in the alloy. These improved ductility at high temperatures for hot rolling, etc. The sensitising range of 750°F to 1650°F made welding "tricky" leading to intergranular corrosion. Because of this columbium was introduced in Alloy-20 to stabilize it. If columbium is present, welding has to be done with great care due to longitudinal weld shrinkage which results in thermal cracking. Alloy-20 with carbon as low as 0.04 per cent without columbium has been developed. This is less susceptible to intergranular attack but finds use only in piping and tubes. In sheet forms Alloy-20 with columbium is preferable because of its high machinability and resistance to breakdown in the sensitizing range when fabrication is done by consumable electrode method (hand welding).

Corrosion of Stainless Steels

Stainless steels perform best under oxidising conditions which are harmful to steel and many of the nonferrous alloys and metals. The chromium present in alloyed form above 12 per cent makes the alloy passive, much the same way as pure chromium, by formation of a thin oxide film which has high resistance to chemical attack. To be effective, the oxide film should form on a clean base-metal surface. In certain cases, as with nonoxidising environment, stainless steel is superior to steel because of its slow rate of solubility ; alloying elements such as nickel, molybdenum and chromium to a lesser extent, confer high resistance to dissolution. Nickel which is less reactive than iron or

chromium, supplements chromium in resisting corrosion by both oxidising and reducing solutions. Nickel, by changing the steel to the austenitic type also improves the structure and mechanical properties. Under severely reducing conditions, as in the case of hot sulphuric acid handling, the proportion of nickel should be considerable and in excess of chromium. The austenitic structure is maintained during the heating and cooling to which the steel is subjected during fabrication processes. Other elements such as molybdenum and columbium confer special corrosion resistant properties. When these are present, the nickel content of the alloy is increased still further to prevent undesirable phase changes during hot working, annealing, etc.

Corrosive attack on stainless steels may occur in any of the following ways : General corrosion ; pitting ; intergranular corrosion ; contact corrosion (galvanic) ; and stress corrosion cracking.

General corrosion : This is characterised by even attack on the entire surface. The formation of a passive oxide film or skin on the stainless steel surface prevents any attack leading to "rusting." However, even the straight chromium and chromium-nickel steels can progressively rust in presence of corrosive salt or sea water because of the chloride content.

Pitting : This type of attack is characteristic of halide solutions (chlorides, iodides, bromides and fluorides) and is concentrated on small areas which show fine crevices and fissures. The attack can be at lap joints and at packing joints, which are usually not aerated or insufficiently aerated, and thus tend to lose passivity. Severe pitting and shortening of equipment life can result. In certain cases, types 316, 317, 316L, and low-carbon 317 stainless steels are satisfactory to counteract this effect.

Intergranular corrosion : This form is characteristic in ferritic and austenitic steels. Ferritic steels change their grain structure when heated to temperatures above 1000°C (1820°F) a temperature found along welding bead. This applies to austenitic stainless steels when heated to temperatures of 450°C (840°F) to 900°C (1650°F), a temperature found at a distance of 1/4 to 3/4 in. along the welding bead. The austenitic steels which suffer from change of grain structure are those with carbon over 0.06 per cent and with no carbide precipitation inhibitors such as titanium, tantalum or columbium. This appears to be due to chromium starvation at the grain boundaries, since carbon from the grains moves to the boundary and precipitates as chromium carbide. These points become seats of corrosion on subsequent exposure of the material to mild acid solutions.

Weldable grades have been developed by introduction of carbide-precipitation inhibitors or by decreasing carbon content of the alloys below 0.06 per cent. These grades do not need the heat treatment usually done after welding.

Contact corrosion : This action takes place when stainless steels come in contact with other metals in an electrolyte in which case an electrochemical element is created. Galvanic corrosion is significant in the machine and equipment industries wherein stainless steel comes in contact with fittings and structural members made of other metals. In the passive state, stainless steels are very noble, but in some electrolytes the passivity may be reduced such that the potential becomes lower than the contact metals, leading to attack on the stainless steel. Highly dissociated solutions of chlorides are very reactive. In acute cases the direct contact between two metals can be avoided by an insulation at the point of contact. Cathodic protection through sacrificial anodes or by impressed current techniques is also being tried.

Stress corrosion cracking : This leads to penetrating cracks across or between

crystals and is caused by the combined action of corrosive agents and by the locked up stresses in the material. This cracking occurs only in austenitic stainless steels. Salt solutions with low acid concentration, moist salts, lye containing reducing agents, dilute hydrochloric, sulphuric and sulphurous acids, are mainly responsible for stress corrosion cracking. The cracks run at right angles to the direction of stress. They are usually inter-crystalline cracks, branching out considerably on the surface and through the steel. A stress relief annealing at 870°C (1600°F) for two hours and subsequent cooling in air of the finished product helps to reduce the effect.

Corrosion resistance of austenitic Ni-Cr steels : Type 302 and 304 are equal in their resistance to chemical attack, but the former is more susceptible to intergranular attack due to its high carbon content. For construction of welded equipment, stabilized grades of type 347 or 321 or low-carbon 304 are used. Such equipment are suitable to handle nitric acid, nitrates, organic acids and compounds at moderate temperatures in the food, dairy and pharmaceutical industries. The highly alloyed type 309 and 310 are resistant to oxidation and sulphidation at high temperatures. Type 316, 317 steels with molybdenum have a wider passivity range, and greater resistance to pitting attack. They are highly resistant to sulphuric, sulphurous and phosphoric acids at moderate temperatures, metal chlorides, and hot strong organic acids such as acetic, lactic and fatty acids. Intergranular attack by certain corrosives may be met with low carbon 316L, 317L or 316 Cb types. Austenitic steels are succeptible to pitting and stress corrosion cracking in the presence of halide solutions. Ferritic stainless steels are less susceptible but they crack due to hydrogen embrittlement in presence of nascent hydrogen.

Fabrication

Stainless steels have excellent workability but their higher strength requires greater power in forming operations. They are also work-hardened and there is a greater spring back during forming operations. Free-machining grades are available for bolting material. The weldability of austenitic stainless steels is excellent and the resulting weldments, joints and beads are strong and ductile. Sheets in thicknesses upto 1/8 in. are generally welded with oxyacetylene, atomic hydrogen, or inert gas methods. The latter method facilitates polishing and gives smoother weld beads. Sheets and plates over 1/4 in. thickness are welded by coated electrode metal arc method, the inert gas consumable electrode process or submerged arc process. Intermediate thicknesses can be welded by all methods. Welding electrode must be similar to parent metal chemically and metallurgically. A butt welded joint is prefered from the standpoints of strength, vibration fatigue resistance and ease of grinding and polishing.

Finishes

Stainless steels can be ordered with several finishes directly from the mills and can be further polished to desired ranges. The standard mill finishes are tabulated below (Table V).

Table V. Stainless Steel Finishes

No.	Description
1	Annealed and pickled; frosty and bright
2D	Dull cold rolled, annealed and pickled
2B	Bright rolled after annealing and pickling
4	Standard polished surface (with wheel coated with 120-150 grit emery)
6	Soft lustre imparted by tampico brushing subsequent to No. 4 finish
7	"Commercial mirror finish" obtained with fine emery coated abrasives (250 grit with grease), high lustre

S. S. Cladding

A clad plate is a composite plate consisting of two or more different metals

permanently and metallurgically bonded at the interface by high temperature and pressure ; it is generally produced by rolling on a plate mill. Clad steels upto 3 in. thickness with interface bond shear strength over 20,000 p.s.i. are available. The backing plate is flange or firebox quality steel, with ten or twenty per cent cladding thickness of the base metal. In this type of continuous metal to metal cladding, the heat transfer properties are better than in lined steels.

In fabrication, great care must be taken to maintain the integrity of the clad steels, particularly during welding operations. The clad material should be protected from scratching or gouging ; pickling, grinding and polishing must be carefully performed to avoid penetration of the cladding. The stainless clad steel can be cut without flux injection, using oxy-acetylene flame. It can be cut from cladding side by use of powder flux injection or with special electrodes through the centre of which a flow of oxygen is directed. The weld surfaces are ground by bakelite-bonded alumina wheels, without tilting, to prevent cutting through the cladding. These clad steels can be stress-relieved by heat treatment. Stabilized S. S. grades are also used for cladding steel. S. S. clad steels are of particular interest in the fabrication of fermentor vessels of large capacities for aerobic fermentation, because of the lower cost as compared to solid alloy.

Design of Stainless Steel Equipment

The designer must select suitable alloys for corrosion resistance under the known service conditions, determine the proper surface finish, specify heat treatment, and make provision for extra thickness of metal wall to compensate for corrosion losses during the life of the equipment, etc. Hence the designer, in approaching the problem of design in stainless steel, must

(1) choose the proper alloy for the service conditions ;

(2) decide corrosion allowance depending on whether the corrosion rate is predictable or indeterminate ;

(3) specify stabilized grades where sensitization of the material is expected during welding, etc. ;

(4) specify higher finishes and special surface conditions where necessary. (Scale is removed with nitric acid and carbon deposit can be removed mechanically with stainless steel wool; oil and grease can be removed by solvent, etc.) ;

(5) avoid crevices, and sharp corners in the design of the vessel, to eliminate the seats of corrosion. Radii are generally specified as large as possible to facilitate cleaning ;

(6) minimize or avoid contact between dissimilar metals ;

(7) allow for easy assembly and access where interior valves and strainers and other parts of the equipment are to be cleaned and/or sterilized, as well as for maintenance ;

(8) specify a practice of good aeration and agitation of corrosive media to avoid accumulation of deposits on metal surfaces which lead to formation of differential oxygen or electrolyte concentration cells ;

(9) avoid locked stresses remaining from fabrication steps by stress relieving through annealing. (This is very important when the corrosive chemical is "stress corrosion cracking" causing type) ;

(10) design all heating gadgets to avoid possibilities of overheating or burning the metal ; and

(11) specify passivation of stainless steel when it is likely to be exposed to reducing conditions. (Designer might specify that the entire stainless vessel be passivated if experience dictates the necessity).

Conclusion

Stainless steel combines mechanical strength with heat and corrosion resistance which no other commercial metal can match. The more the experienced the designer, fabricator, user and metallurgist with the performance of stainless steels, the greater will be their reluctance to make general recommendations. It is not that data is lacking, but rather that environment and service conditions vary so widely that a final choice should be made only after evaluating all of the stainless steels and modifications that are available.

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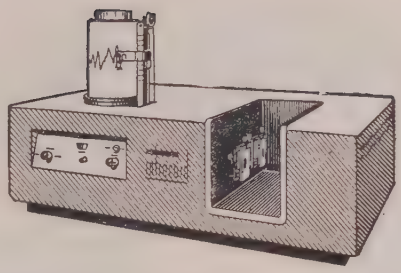

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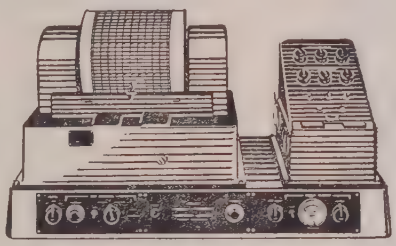

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Influence of some factors on Penicillin Titres in Industrial Fermentors: A Statistical Study

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THE factors influencing penicillin titres have been discussed by Johnson[1] and Ganapathi.[2] Gaden[3] has indicated the most critical factors that govern cell-medium interaction. In industrial fermentation of penicillin, there is but a small variation from the experimental conditions. The amount of antibiotic synthesized at various hours and its peak concentration obtained, however, are found to vary between fermentations. An exploratory investigation was, therefore, undertaken to find an explanation for this variation.

In a batch of fermentations with *Penicillium chrysogenum* HA-3 strain in fermentors of similar design, the penicillin titres and the corresponding values of age, oxygen concentration in the broth, concentration of mycelium, specific uptake rate of oxygen by the mycelium, specific oxygen diffusion rate into the broth, percentage of mycelial nitrogen, resistance of the broth filtrate and percentage of ash of the mycelium, were measured. The relationship between the penicillin titres and the other factors was estimated by multiple correlation analysis. The results obtained are discussed in this paper.

Materials and Methods

The concentration (C_i), the quantity (C_m), specific uptake rate (k_r) and the specific diffusion rate (k_d) of oxygen were estimated by the methods descrideb in earlier communications.[4,5]

Estimation of the Ash Content of the Mycelium

Samples of mycelium obtained from the fermentors were powdered in porcelain mortar and preserved in air tight bottles. Silica crucibles of 25 ml. capacity were cleaned with chromic acid, washed with double distilled water and alternately heated at 700°C for 1 hr. cooled in a desiccator and weighed until the weight remained constant. Mycelium samples (0.1-0.2 g.) dried at 100°C for 2 hr. were transferred to the crucibles, heated slowly to 700°C, kept at that temperature for nearly 16 hr. then cooled in a desiccator and weighed. The process of heating was repeated until the ashing was complete as indicated by no loss in weight. Ashing was complete when the samples were kept at 500°C for 48-72 hr. or at 700°C and 900°C for less than 24 hr. The difference in ash content by ashing at 700°C and 900°C was less than 5 per cent. As heating at 900°C reduces the effective life of crucibles a temperature of 700°C was preferred for ashing. The ash obtained was nearly white.

Estimation of Total Nitrogen in the Mycelium

Mycelium samples (0.1g.) dried at 100°C for 2 hr. were digested with conc. sulphuric acid (2 ml.), treated with hydrogen peroxide till the solutions were colourless, and diluted to 100 ml. A standard and a blank were run under similar conditions.

Three methods were tried for the estimation of the concentration of ammonia in the solutions. In the first method, the solutions (5 ml.) placed in the vacuum jacketted distillation apparatus of Parnas and Wagner[7] were decomposed with 40 per cent sodium hydroxide solution (10 ml.) and distilled. The distillate was absorbed in 2 per cent boric acid solution (10 ml.). To ensure that the same amount of heat was transferred to the sample, the distillation was carried out till the distillates measured the same volume in each experiment. The volume required for complete recovery of ammonia was determined by experimenting with an ammonium sulphate solution containing approximately the same amount of nitrogen as the sample solutions. The distillate obtained was directly titrated with 0.01M hydrochloric acid using Tashiro indicator.

The second method consisted in nesslerising[8] the digests directly and comparing their optical densities at 490 mμ with those of standard solutions of nitrogen made with the blank solution.

In the third method, the distillates were diluted, nesslerised, again diluted and the optical densities of the solutions compared with standard solutions made with 2 per cent boric acid solution.

The discrepancies between the values for the percentage of nitrogen obtained by the three methods were tested statistically by the student's *t* test[9]. With twenty-five samples of mycelium of different ages collected from four fermentations, the values of student's *t* indicated that the variations between the values obtained by the three methods were not significant at 5 per cent probability level. The first method was, therefore, followed in this investigation.

Measurement of the resistance of the broth Filtrate

The broth was filtered through nylon cloth and a conductivity cell with a resistance capacity of 0.855/cm. supplied with Metrohm conductometer was used for the determination of the resistance of the filtrate at 24°C.

Method of Fermentation

The 19 fermentations studied were carried out as indicated below in six different fermentors of equal capacity, similar design and agitation-aeration systems:

Fermentor No.	*Fermentation No.*
1	1, 2, 3.
2	4, 5.
3	6, 7, 8, 9.
4	10, 11, 12.
5	13, 14, 15, 16, 17.
6	18, 19.

The production medium was inoculated with seed mycelia grown by inoculating *P. chrysogenum* spores into nutrient medium in seed tanks and aerating and agitating for nearly 50 hr. at 24°C. The composition of the medium in the seed vessel was (in percentage): cornsteep liquor 3.13, sucrose 1.85, sodium nitrate 0.275, calcium carbonate 0.314, potassium dihydrogen phosphate 0.0263, magnesium sulphate 0.00563, and sodium hydroxide 0.0387. Peanut oil with 3 per cent octadecanol (0.0988 per cent) was added as antifoam. The volume of the inoculum was 7.7-8.0 per cent of the total volume of the broth. The cornsteep liquor-lactose medium in the fermentors contained (in percentage): cornsteep liquor 0.93, peanut meal 2.79, lactose 3.86, calcium carbonate 0.558, sodium sulphate 0.111, phenyl acetamide 0.042, magnesium sulphate 0.0055, sodium hydroxide 0.0358; peanut oil with 3 per cent octadecanol 0.1135, added as antifoam. The strain HA-3 is a derivative of *Penicillium chrysogenum* Wis. 51-20 strain. The fermentation was carried out at 24°C for 83-107 hr. till maximum concentration of penicillin was obtained.

The method generally followed in fermentation is described above. There were, however, some variations : In fermentations 4 and 11, the production media were seeded with the broth (5.5-5.6 per cent) from other fermentations ; an equal quantity of glucose replaced sucrose (1.85 per cent) in the seed media employed for the preparation of inoculum in fermentations 8 and 17 ; the quantity of magnesium sulphate in the seed medium was 0.00688 per cent in fermentations 5, 8, 9, 16 and 17. The variation in the production medium are given below : In fermentations 1, 2, 4, 13, 14, 15 the peanut meal content of media was 2.3 per cent as against 2.79 per cent in the others, and it was enhanced to 3.26 per cent in fermentation 6 ; the amount of lactose was 3.49, 3.63 and 3.70 per cent in fermentations 4 and 13, 14 and 1 respectively ; sucrose (0.91 per cent) partially replaced lactose in fermentation 15.

The time and quantity of the precursor addition was the same and the maximum variation in the total quantity of the broth at the end of the fermentation cycles was 11.3 per cent. The amount of oil containing antifoam varied from 0.46-1.86 per cent in the different fermentations. The type of mycelium produced in these processes was a mixed type containing pellets. The pH of the production medium was about 6.2 rising to about pH 7 during the penicillin producing phase of the fermentation.

Multiple Correlation Analysis

The application of multiple correlation analysis is presented by Gore.[10] The penicillin titres (Y_o) in four fermentations and the corresponding magnitudes of the factors (X_1, X_2 ....X_8) mentioned above are indicated in Table I. The values of the different variables were measured in all fermentations and the relationship between penicillin titres (Y_o) and each factor (*e.g.* X_1) was estimated by calculating the primary correlation coefficients (r_o) on the basis of formula (1) where N, the number of measurements, is 94.

$$r_o = \frac{\Sigma XY - \frac{\Sigma X \Sigma Y}{N}}{\sqrt{\left(\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right)\left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right)}} \quad \ldots . (1)$$

The magnitudes of the coefficients are presented in Table II. The statistical significance of these correlation coefficients was tested at a level of P=0.01 by using the tables of Fisher and Yates.[11] The number of pairs of data available was 94 and therefore, for a total correlation the number of degrees of freedom n was equal to N—2. The magnitude of the correlation coefficients when n=80 and n=90 were reported by Fisher and Yates[11] to be 0.2830 and 0.2673 respectively. The values of all the coefficients except r_{05} were significant. Johnson[1] and Ganapathi[2] have pointed out that an appropriate combination of several conditions was necessary for obtaining good yields of penicillin. The influence of the interaction between the factors was, therefore, calculated on the basis of the interfactor correlation coefficients presented in Table III evaluated by equation (1). The standardized partial regression coefficients or β coefficients were calculated on the basis of simultaneous equations (Table IV). The fraction of the total variance of the penicillin titres which was attributed to each factor is given by the product of its β coefficient and its primary correlation coefficient (Table V). This product defines the relative importance of each factor. The products of $r_{03}\beta_3$ and $r_{07}\beta_7$ alone were positive. All factors other than X_3 and X_7 were, therefore, eliminated in the evaluation of β coefficients by simultaneous equations (Table VI). The recomputed values of the β coefficients and the total variance of the penicillin titres indicated by the two factors are given by the sum of the products (Table VII). The β coefficients were employed to determine

TABLE I

DATA FROM FERMENTATIONS

Strain—HA-3 in fermentor 3

No. of Fermentations	Penicillin u/ml x 10^{-2}	Age (hr.)	Oxygen C* x 10^4 (g. moles/ml.)	Myelium percent (g/ml.)	k_r x 10^4 g moles of 0_2/ (g of mycelium) (hr.)	k_d x 10^4 g moles of 0_2/ (ml.) (hr.) (atm.)	Mycelial nitrogen percent. (w/w)	Resistance (ohms x 10^{-2})	Ash percent. (w/w)
	Y_0	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
6	8.20	38.00	3.25	2.83	3.73	0.48	7.03	1.90	16.60
	13.60	45.00	8.05	3.27	4.87	0.97	6.37	2.00	15.48
	22.80	62.00	4.98	3.95	4.08	0.68	6.00	2.30	13.95
	27.70	69.00	3.70	4.28	5.27	0.89	5.62	2.30	13.41
	35.70	86.00	1.78	4.60	2.77	0.40	5.28	2.50	13.15
	37.59	93.00	13.18	4.16	3.46	0.91	5.54	2.45	14.45
7 ..	9.60	38.50	12.79	2.73	4.94	1.17	5.65	2.10	15.78
	14.00	45.50	9.85	3.01	4.63	0.97	5.94	2.10	15.86
	22.50	62.50	12.31	3.53	4.30	1.02	5.60	2.50	15.38
	26.50	69.50	11.54	3.61	3.84	0.83	4.94	2.50	14.30
	30.00	86.50	15.07	3.71	3.12	0.79	5.51	2.85	14.04
	32.50	93.50	14.55	3.49	3.81	0.84	5.11	2.85	15.89
8 ..	11.40	40.50	14.79	2.97	3.26	1.54	6.87	2.20	23.27
	16.00	47.50	9.66	3.37	4.18	1.00	6.08	2.20	15.97
	25.00	64.50	17.22	3.92	4.03	2.44	5.03	2.60	14.78
	28.80	71.50	17.22	4.14	3.16	1.81	4.85	2.60	14.39
	32.30	88.50	20.24	3.86	3.21	2.80	5.21	3.10	15.36
	33.80	95.50	21.54	3.54	2.98	2.31	4.98	3.00	16.03
9 ..	13.30	48.00	13.28	3.06	4.54	1.49	6.08	2.10	18.86
	20.00	55.00	11.93	3.36	3.84	1.19	5.78	2.15	17.30
	28.10	72.00	15.55	3.76	3.59	1.79	5.00	2.50	16.81
	30.00	79.00	17.91	3.70	2.85	1.35	4.93	2.55	16.19

slope constants b_3 and b_7 in the multiple regression equation (2).

$$Y_o = b_3X_3 + b_7X_7 + A \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where A is the intercept constant. The slopes b_3 and b_7 were calculated by equations similar to (3) :

$$b_3 = \frac{\beta_{3so}}{s3} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3),$$

where so is the standard deviation of Y_o observations and s_3 is that of the factor X_3 (Table II). The standard deviation was obtained by formula (4) :

$$s = \sqrt{\frac{\Sigma_1^N X_i^2}{N-1} - \frac{(\Sigma_1^N X_i)^2}{N(N-1)}} \quad \ldots\ldots (4)$$

The intercept constant A of the equation (2) was obtained by substituting the values of $\bar{X}_3$, $\bar{X}_7$ and Y_o which are the averages (Table II) of the measurements of the factors X_3, X_7 and Y_o estimated by equation (5) :

$$\bar{X} = \frac{\Sigma_1^N X_i}{N} \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The multiple regression equation obtained was

$$Y_o = 10.873X_3 + 16.922X_7 - 56.463. \quad (6)$$

The reliability of the estimated values was derived from the standard error of the estimate Sy (est) on the basis of the formula (7) :

$$Sy_{o(est)} = sy_o \sqrt{(1-R^2)\frac{(N-1)}{(N-k-1)}} \quad (7)$$

where k is the number of independent factors which was two in equation (6). The actual values of the penicillin titres will be within plus or minus two standard errors of the estimate in 95 times out of 100. All the values will lie, however, within three standard errors of the estimate.

TABLE II

AVERAGES (X), PRIMARY CORRELATION CO-EFFICIENTS (r_o) AND STANDARD DEVIATIONS (s)

Factor	X	r_o	s
Y_0	21.1144	—	s_0 = 8.8501
X_1 ..	63.1303	r_{01} = + 0.92403	s_1 = 18.1250
X_2 ..	12.7097	r_{02} = + 0.37186	s_2 = 4.5439
X_3 ..	3.3933	r_{03} = + 0.78053	s_3 = 0.5012
X_4 ..	4.1155	r_{04} = − 0.29067	s_4 = 1.0887
X_5 ..	1.3541	r_{05} = + 0.09088	s_5 = 0.6768
X_6 ..	5.4355	r_{06} = − 0.54806	s_6 = 0.8728
X_7 ..	2.4000	r_{07} = + 0.72819	s_7 = 0.2827
X_8 ..	15.8668	r_{08} = − 0.44685	s_8 = 2.3505

TABLE III

INTERFACTOR CORRELATION COEFFICIENTS

r_{12} = + 0.39614	r_{23} = + 0.12627	r_{36} = − 0.63236	r_{67} = − 0.58853
r_{13} = + 0.76757	r_{24} = − 0.33439	r_{37} = + 0.30493	r_{68} = + 0.62109
r_{14} = − 0.43570	r_{26} = − 0.32220	r_{38} = − 0.70354	r_{78} = − 0.21358
r_{16} = − 0.61050	r_{27} = + 0.64788	r_{46} = + 0.32183	
r_{17} = + 0.73487	r_{28} = + 0.12156	r_{47} = − 0.48474	
r_{18} = − 0.47372	r_{34} = − 0.46690	r_{48} = + 0.07408	

TABLE IV

SIMULTANEOUS EQUATIONS FOR ESTIMATING β COEFFICIENTS

r_{01}	$= \beta_1$	$+ r_{12}\beta_2$	$+ r_{13}\beta_3$	$+ r_{14}\beta_4$	$+ r_{16}\beta_6$	$+ r_{17}\beta_7$	$+ r_{18}\beta_8$
r_{02}	$= r_{12}\beta_1$	$+ \beta_2$	$+ r_{23}\beta_3$	$+ r_{24}\beta_4$	$+ r_{26}\beta_6$	$+ r_{27}\beta_7$	$+ r_{28}\beta_8$
r_{03}	$= r_{13}\beta_1$	$+ r_{23}\beta_2$	$+ \beta_3$	$+ r_{34}\beta_4$	$+ r_{36}\beta_6$	$+ r_{37}\beta_7$	$+ r_{38}\beta_8$
r_{04}	$= r_{14}\beta_1$	$+ r_{24}\beta_2$	$+ r_{34}\beta_3$	$+ \beta_4$	$+ r_{46}\beta_6$	$+ r_{47}\beta_7$	$+ r_{48}\beta_8$
r_{06}	$= r_{16}\beta_1$	$+ r_{26}\beta_2$	$+ r_{36}\beta_3$	$+ r_{46}\beta_4$	$+ \beta_6$	$+ r_{67}\beta_7$	$+ r_{68}\beta_8$
r_{07}	$= r_{17}\beta_1$	$+ r_{27}\beta_2$	$+ r_{37}\beta_3$	$+ r_{47}\beta_4$	$+ r_{67}\beta_6$	$+ \beta_7$	$+ r_{78}\beta_8$
r_{08}	$= r_{18}\beta_1$	$+ r_{28}\beta_2$	$+ r_{38}\beta_3$	$+ r_{48}\beta_4$	$+ r_{68}\beta_6$	$+ r_{78}\beta_7$	$+ \beta_8$

TABLE V

PRODUCTS OF PRIMARY CORRELATION COEFFICIENTS AND β COEFFICIENTS

Factor	r_0	β	$r_0\beta$
X_1	+ 0.92403	— 1.61822	— 1.49529
X_2	+ 0.37186	— 0.48562	— 0.18058
X_3	+ 0.78053	+ 2.95636	+ 2.30753
X_4	— 0.29067	+ 1.13695	— 0.33048
X_6	— 0.54806	+ 0.68912	— 0.37768
X_7	+ 0.72819	+ 2.48901	+ 1.81247
X_8	— 0.44685	+ 0.94488	— 0.42222

TABLE VI

SIMULTANEOUS EQUATIONS FOR RECOMPUTING β COEFFICIENTS

r_{03}	$= \beta_3$	$+ r_{37}\beta_7$
r_{07}	$= r_{37}\beta_3$	$+ \beta_7$

TABLE VII

PRODUCTS OF PRIMARY CORRELATION COEFFICIENTS WITH THE RECOMPUTED β COEFFICIENTS

Factor	r_0	β	$r_0\beta$
X_3	+ 0.78053	+ 0.61574	+ 0.48060
X_7	+ 0.72819	+ 0.54044	+ 0.39354
	$R^2 = r_{03}\beta_3 + r_{07}\beta_7$	=	0.87414

Results and Discussions

Fermentations of penicillin and streptomycin are different from other processes as the products synthesized do not arise from energy metabolism but are independently elaborated or accumulated by cells, and the major products of energy metabolism are carbon dioxide and water. The process scale-up in such fermentations is difficult as adequate knowledge of the number of reactions, the range of variation of the various physical and chemical factors involved and their influence on the synthesis of the product are not available. As emphasized by Gaden[3], attempts made hereto have been restricted to the scaling-up of the reactor and its agitation-aeration system. He has questioned the validity of the general scale-up procedure for aerobic fermentation on the basis of equivalent oxygen absorption suggested by Bartholomew *et al*[14, 15] and by Karow *et al*[16]. The microscopic viewpoint of the fermentation process put forward by Donovick[12] supports Gaden.[3] Donovick[12] has indicated that agitation is not primarily concerned with oxygen transfer and successful penicillin fermentations are obtained under conditions of low efficiency of oxygen transfer. He has stressed that agitation not only helps in oxygen transfer to the broth but also influences the concentration gradient of all solutes in the immediate vicinity around the cell. The gradients of the

nutritional substances moving into the cell and all waste products extruded out of the cell govern their diffusion coefficients and hence their rates of diffusion into and out of the cell. Ultimately, the reaction rates within the cell and hence the kinetics of formation of penicillin is governed by diffusion rates of the various substances. Agitation, therefore, is related to the kinetics of the process. Gaden[13] has discussed the major process variables on growth and antibiotic formation. He has shown that with pH control, the rates of metabolism and penicillin formation can be regulated for a long time.

In the absence of a logical method for process scale-up on the basis of chemical reactions and other factors mentioned above, the scale-up procedure consists only in reproducing completely in equipment of any size, all ambient conditions for cell-medium interaction which are known to be optimum for achieving maximum productivity. This is a practical approach to scale-up in contrast to that of Bartholomew *et al*[14, 15] and Karow *et al*[16] in which oxygen absorption within the medium alone is the guiding principle. Prerequisite in adopting such a procedure is, however, the knowledge of the factors and their interaction which affect productivity.

In processes where there are many factors which influence productivity, the investigation is restricted to the observation of relatively few factors as in the present investigation, because it is difficult to measure all of them. Besides, when the effect of a large number of independent variables on the dependent variable such as penicillin titres is to be investigated, graphical methods are inadequate. Recourse has, therefore, to be taken to statistical methods[6] as they can be applied to conditions where controlled experimentation is inexpedient. A comparison between the titres obtained on the basis of the multiple regression equation (6) and the experimental values is shown in Fig. 1, which indicates appreciable agreement between the two sets of values.

The variation in the type, age and amount of the inoculum do not appear to influence the penicillin titres. The amount of antifoam oil added varied considerably, with a maximum amount (1.84 per cent) in fermentation 19 and minimum (0.46 per cent) in fermentation 11. Deindoerfer and Gaden[17] found that the addition of a mixture of Alkaterage C and lard oil decreased the oxygen absorption in the medium. A similar effect of the concentration of antifoam oil can be seen from the data given in Table VIII in which the values of the the solubility of oxygen, its concentration during fermentation and the diffusion rate coefficient into the medium are higher in Fermentation 11 where minimum amount of the antifoam was used. Penicillin titres were not, however, influenced by this variation in oxygen diffusion rate. In fact they were higher by 13 per cent at the same age in Fermentation 19, probably indicating that the variation of this factor is not critical in the process. The same method was followed for the sterilization of the medium and conditions such as temperature,

TABLE VIII

EFFECT OF ANTIFOAM OIL ON THE CONCENTRATION OF OXYGEN IN THE BROTH, ITS SOLUBILITY IN THE BROTH FILTRATE AND THE DIFFUSION RATE CONSTANT AT VARIOUS HOURS OF FERMENTATION

Fermentation Number	Age (hr.)	$C_i \times 10^6$ gram moles of O_2/ml.	$C^* \times 10^6$ grams moles of O_2/ml.	$k_d \times 10^4$
19	14	0.1889	0.10860	0.08095
	19	0.1900	0.07399	0.15400
	36	0.1923	0.05797	0.48960
	43	0.1914	0.07943	0.93760
	60	0.2021	0.06434	0.58110
	67	0.2054	0.11280	0.96740
	84	0.2189	0.10300	0.64890
	91	0.2254	0.06256	0.52080
11	12	0.2007	0.13330	1.5990
	19	0.2194	0.06317	0.3531
	36	0.2196	0.06507	0.5985
	43	0.2146	0.09410	0.9754
	60	0.2278	0.06717	0.8574
	67	0.2428	0.06393	0.9191
	84	0.2658	0.07938	0.6270
	91	0.2684	0.14070	0.8706

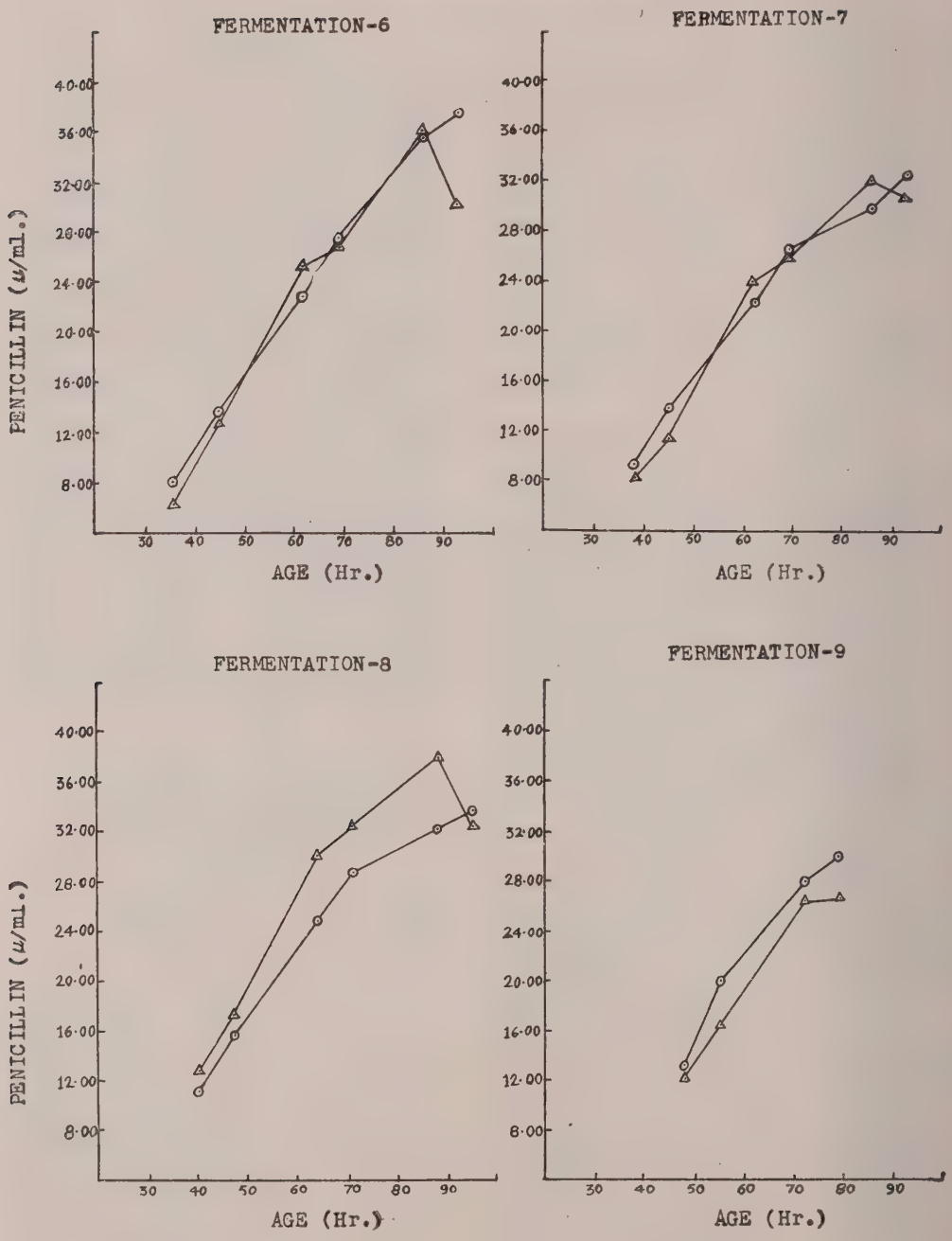

Fig. 1. Comparison of experimental and estimated values of penicillin titres.

o——o Experimental values Δ——Δ Estimated values

pressure, oxygen supply and agitation were the same in all the experiments. The variation in pH of the broth from pH 7 was $\pm$ 0.1 pH units. Lactose was present in trace amounts and at the time of many observations no lactose was found. The two factors were not, therefore, taken up for analysis.

The values of standard deviations (Table II) indicate the spread of the data from the average values. This deviation is useful in estimating the magnitude of change in the process variables under a given set of experimental conditions. Due to the characteristic property of this deviation, approximately 68.7 per cent of the values lie between plus or minus one standard deviation from the average. Ninety-five per cent of the values are, however, within plus or minus two standard deviations from the average. For the factor X_3 which is the percentage of mycelium in the broth the deviation is 0.5012. Good crop of mycelium which is not multiplying is a condition associated with high productivity in the batch process. The deviation is a measure of the variation of the quantity and multiplication of mycelium and is useful in comparing one set of conditions with the other. The average 3.3933 can be employed similarly. The high value of 4.5439 for the deviation of X_2, the concentration of oxygen in the broth, indicates a marked variation from the average concentrations of 12.7097×10^{-4} g moles/ml. during fermentation. Since sufficient amount of dissolved oxygen is required for good penicillin production, the average and standard deviations can be employed for comparison as mentioned above. The average 4.1155 g. moles of oxygen/(g. mycelium) (hr.) for k_r, the specific uptake rate of oxygen by the mycelium (factor X_4) can be employed for calculating the oxygen demand of the broth which in the present investigation was nearly one-third of the volume of the air supplied per minute.

The primary correlation coefficient measures the extent of relationship between each factor and the penicillin titres. The coefficient has no dimensions and its value ranges from $+1.0$ to -1.0. A value of 1.0 for the coefficient indicates a perfect proportionality between the titres and the factor, and the value of -1 shows a perfect inverse relationship (*e.g.*, r_{o4}, r_{o6} and r_{o8} in table II). If the value of the coefficient is zero, no relationship exists between the two factors. The correlation coefficient is statistically significant if it has not been obtained by chance. The test of significance is carried out by estimating the probability with which the calculated coefficient is obtained from random samples. If the probability is low, it indicates that the calculated value of the coefficient has not been obtained by chance and the observed correlation has real significance in the population of data studied. The application of the test of significance has already been shown earlier in this paper.

In equation (6) only two factors X_3 and X_7 which are the concentrations of mycelium in the broth and the resistance of the broth filtrate explain 87.414 per cent of the total variance of penicillin titres, the first factor accounting for 48.060 per cent and the second for 39.354 per cent. Further, the titres are directly proportional to the variation of these factors in equation (6) suggesting that a rise in the magnitudes of these process variables enhance the yield of penicillin. The equation suggests that causes for the variation of the important process variables, quantity of mycelium and the resistance of the broth filtrate during the penicillin producing phase of the fermentation, should be investigated. It has already been noted that a good crop of mycelium is associated with successful penicillin fermentation. The analysis, therefore, recommends that attempts should be directed to produce the same quantity of mycelium to eliminate variation in titres between fermentations. As reciprocal of the resistance is the conductance of the solution, a fall in conductivity of the broth filtrate, influences penicillin titres. The broth filtrate contains, ions of the salts

incorporated in the medium, ions derived from the cornsteep liquor, peanut meal and other organic substances. The hydrogen and hydroxyl ions have maximum ionic conductivities. The pH during the study was maintained at about 7, the concentrations of both the ions were, therefore, equal. Equation (6) indicates loss of hydrogen ions and other ions in the medium which increased the resistance of the broth filtrate which favours better yields. The maintenance of pH at a level higher than 7 may, therefore, enhance the production of penicillin.[13] In the present studies the pH during the growth phase was over 6.2 and varied between 6.9 and 7.1 during the penicillin producing phase. The variation in the penicillin titres observed may, therefore, be due to the factors solicited by the analysis. The conclusions reached are valid only for the factors considered in this analysis. The analysis is based on the assumption that the relationship between penicillin titres and other variables is linear. The method can be refined if necessary by exploring curvilinear relationships.

Fermentation process kinetics is primarily concerned with the rates of reactions in large scale production and particularly with the effects of process variables on them.[13] As it is easy to calculate the rates of reactions, the methods outlined in this paper, which indicates the effects of process variables may, therefore, be applied to the study of the fermentation process kinetics.

ACKNOWLEDGEMENT

The authors are grateful to Dr. K. Ganapathi for suggesting the problem, and to Dr. M. J. Thirumalachar and Dr. D. Ghosh for their interest in the investigation.

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Production of *Penicillium* Mutants by Ultrasonic Waves and Shortwave Diathermy

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THERE is a vast and growing literature on the use of ultraviolet and X-rays as mutagenic agents in micro-organisms, but the use of ultrasonic waves and diathermy has until recently been restricted to physical medicine and clinical or biochemical techniques. Recently ultrasonic waves have been tried as mutagenic agents in *Streptomyces griseus*,[1] bacteria[2] and protozoa.[3] Zuckermann[4] records that the oak-wilt fungus was insensitive to certain ultrasonic dosages. As far as the authors are aware, there is no record of the use of various forms of diathermy in inducing mutations. On the basis of the survival values in preliminary experimentation we considered these agents to be more suited to induce delicate variations in the cell than the rather drastic effects of ultraviolet and X-rays.

In a general programme of improvement of penicillin yields by mutation and selection of better yielding strains of *P. chrysogenum* we have successfully used both ultrasonics and diathermy in obtaining not only high penicillin yielders but also morphological mutants different in growth pattern and colour of spores in comparison with the parent population.

Materials and Methods

Spore suspensions of a strain of *P. chrysogenum* (designated HA-6 in this laboratory) were made in sterile water from 7 day old cultures on a suitable sporulating medium. The spore colour of the parent population was "capuccine buff".[5] The time of exposure to ultrasonics was varied keeping the variac reading and the current constant. The treatment details are given in Table I. The temperature of the treatment cell was higher than that of the room but the temperatures were noted and control spores treated similarly. After treatment the control spores and treated spores were plated on solid media for colony development and study. Different dilutions were used to recover isolates. The conditions of media volume and incubation were same in all platings. At the end of seven days observations were made on the population growth pattern, spore colour, type of colony and spore germination. Spores collected from the isolates were inoculated in liquid media and shaken flask fermentations carried out using the usual lactose-cornsteep-calcium carbonate medium for penicillin production. Penicillin was estimated by the iodometric method. Parallel soil cultures and reference slants were made simultaneously for preservation of the promising variants.

TABLE I.
ULTRASONIC TREATMENT OF HA-6 SPORES
(Spore concentration 25 mil. /ml.)

Time (min.)	Temp. (°C) in Treatment cell	Variac reading	Current (milliamp.)	No. of isolates
4 ..	35	240	2.3	48
8 ..	38	240	2.3	51
12 ..	38	240	2.3	49

Growth rates of 100 colonies each from the parent and the treated populations were studied. All the isolates were grown under similar conditions and the colony diameter measured at the end of seven days and classified into a frequency distribution. Table II.

TABLE II.

COMPARISON OF GROWTH PATTERN OF THE PARENT AND TREATED POPULATIONS

Colony diameter mm.	Growth frequency distribution			
	Control	Mean	Treated	Mean
11-13	3		—	
13-15	33		—	
15-17	53		1	
17-19	11	15.37	16	
19-21	—		70	
21-23	—		11	
23-25	—		2	19.94

The diathermy treatment was given in a Siemens Ultratherm 603 apparatus to spore suspensions made as indicated above. The treatment was for varying periods of time till the intensity was high enough to give 100 per cent kill. Details of the treatment and survival data are given in Table III.

TABLE III.

SURVIVAL DATA, IN ULTRA-SHORT WAVE DIATHERMY TREATMENT

Time (min.)	No. of colonies after 4 days	Survival per cent.
Control	650	100.0
5	500	76.9
10	400	61.5
15	200	30.7
20	nil	00.0

It is evident from the tables the treated population shows a faster growth rate than the controls, the mean for the entire treatment being significantly higher than that of the control.

Analysis of penicillin production of the isolates studied, indicated that the majority of the isolates were in the range of the parent control, while 6 per cent gave yields 30 per cent better than the controls and 6 per cent of the isolates gave an equally low yield. Majority of the isolates from the treated population showed spore colours similar to that of the parent except for 1 per cent which were deeper brown and 1 per cent which were completely white but sporing; 6 per cent of the isolates showed sectoring. No mycelial types were recovered. About 25 per cent of the colonies recovered were studied and classified on the basis of spore colour. Table IV summarizes these observations.

TABLE IV.

ANALYSIS OF THE DIATHERMY TREATED POPULATION ON THE BASIS OF SPORE COLOUR.

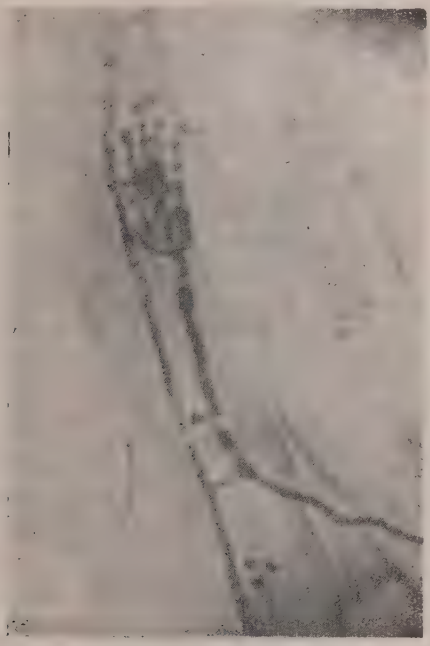
Treatment	No. of isolates	White	Light brown	Deep brown
5 min. ..	80	5	48	27
10 „ ..	80	2	48	30
15 „ ..	92	6	55	31
Control ..	100	1	98	1

As may be seen from the table there is a shift to deeper colouration of spores in the treated population. An analysis of the penicillin production of the isolates indicated 75 per cent within the range of parent population, 20 per cent with yields up to 30 per cent higher and 8 per cent with yields 30 per cent lower and 2 per cent producing negligible quantities of penicillin. The last group is under study as it was derived from a high yielding strain.

In the case of diathermy treatment a number of morphological mutants, white colonies and mycelial types were recovered. The spore colour varied from light to deep brown and a few isolates sporulated within three days in the usual sporulating medium,

without lowering of the penicillin producing capacity.

Interesting types included a series of isolates which showed free anastamoses even between two adjacent conidiophores. This is the first time we have come across a freely anastamosing high yielder. Further studies on the isolates from this strain are in progress.



Photomicrograph showing anastamoses in a diathermy mutant (x 600)

Two other types deserve mention, one of them an extremely poor penicillin yielder was a white sporulating type, with erect growing sparse hyphae from the centre of the colony and another a yellowish green[6] spored one, a type we have also isolated from the nitrogen mustard treatment. Variations among representative populations of these isolates are under study.

From the above studies we would conclude that ultrasonic waves and ultrawave diathermy treatments of spores offer newer methods of producing useful mutants in industrial microorganisms.

ACKNOWLEDGEMENT

We are grateful to Prof. S. K. K. Jatkar and Mr. N. R. Kale of the University of Poona for advice and help in ultrasonic treatment, and to Miss J. M. Maniar, Physiotherapist in the ultrawave diathermy treatment. Our thanks are due to Dr. N. Narasimhachari for the penicillin assays and to Mr. J. D. Adhia for kind encouragement.

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Studies on the Control of Root Rots of some Crop Plants with Nystatin*

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NYSTATIN or fungicidin, a tetraene produced by *Streptomyces noursei* was shown by Hazen and Brown[2] to be a powerful antifungal antibiotic. In addition to its varied uses in the therapy of fungal diseases in human beings, experiments have also been carried out in controlling plant diseases. The antibiotic has been used as a spray against the downy mildew of cucurbits, *Pseudoperonospora cubensis* (Berk and Curt) Rostoz,[1] *Endothia parasitica*(Murr), and *Sclerotium rolfsii* Sacc., *Dipolodia malorum*[8] and many others. In India, Rangaswami and Damodaran[6] showed that the pathogens inciting the anthracnose and wilt of banana (*Gloeosporium musarum* Cke and Mass, and *Fusarium oxysporum* Schlecht. f. *cubense* (E.F.S.) Snyd and Hans.) are inhibited by various concentrations of nystatin. The same authors have shown that nystatin is fungistatic to *Helminthosporium torulosum* (Syd) Ashby.

While numerous reports have been published about the possible uses of nystatin combinations as sprays and dusts for controlling different plant diseases, it became apparent that under the conditions prevailing in India, only seed treatment is practicable, that too, if it has advantage over other chemicals already in use for seed treatment. This is chiefly due to the exorbitant cost of the antibiotic, and other related economic factors. In the present study, the seedling blights incited by four major soil-borne plant pathogens were chosen, and the effects of seed treatment with nystatin in relation to disease incidence was studied.

Materials and Methods

Pure sample of nystatin was obtained by the senior author through the courtesy of Dr. J. C. Mody, Medical Director, Sarabhai Chemical Co., Baroda. One μg of the substance assayed about 2 units. For finding the inhibitory threshold concentration of the antibiotic, solutions were prepared aseptically. In the case of seed treatments, the antibiotic was used directly for seed dressing, with or without solid dilutants depending upon the volume of the seed to be treated.

The four fungal pathogens studied were *Pythium aphanidermatum* (Edson) Fitzp., *Colletotrichum indicum* Dastur, *Rhizoctonia solani* Kuehn and *Macrophomina phaseoli* (Maubl.) Ashby. With the exception of *C. indicum*, the other pathogens are worldwide in distribution and incite damping off and wilts of seedlings of various crop plants.

Studies on the Action of the Antibiotic *in Vitro*

The inhibitory threshold range of the antibiotic was determined by incorporating known quantities of the antibiotic in potato dextrose agar, and seeding them with the

* The trade name of E. R. Squibb and Sons for fungicidin is Nystatin.

spores of the fungus. In the case of Sclerotia of *Rhizoctonia solani* and *Macrophomina phaseoli*, they were also spread on the surface of agar containing the antibiotic. In *Pythium aphanidermatum* 3 mm. discs cut from the agar plate with luxuriant growth were used. The results taken after 120 hr. incubation showed that the inhibitory concentration for each of the fungus tested was as follows :

1. *Colletotrichum indicum* 2 to 2.5 μg/ml.
2. *Macrophomina phaseoli* 8 to 9 μg/ml.
3. *Rhizoctonia solani* 15 to 17 μg/ml.
4. *Pythium aphanidermatum* 1000 μg/ml. had no action.

Microscopic examination of the spores, sclerotia and mycelia of these fungi exposed to different concentrations of the antibiotic were made, and any morphological changes that may be present were noted. In *Colletotrichum indicum*, the falcate spores germinated very rapidly forming a septate mycelium. When 1 μg/ml. of the antibiotic was added, the hyphae became stout and closely septate. When the dosage was above 2 μg/ml. the small germ tubes that were produced as a bulge, burst and all the spore contents extruded. The spores and mycelia produced thus disintegrated. This mode of action was similar to that reported by Walker[9] in the onion smudge fungus *Colletotrichum circinans*. In this case, Walker showed that the protocatechuic acid and catechol compounds diffusing out of the red onion scales caused bursting of the germ tubes thus making the red onion varieties resistant to the fungus. That the same effect is produced by the antibiotic nystatin on another species of *Colletotrichum* is a noteworthy feature.

In the case of *M. phaseoli*, addition of nystatin to the agar medium upto 5 μg/ml. had no effect and the germination of the sclerotia was normal. The mycelial development was very rapid at 28°, and within 24 hours, the entire petri plate was covered with hyphae. Slight retardation of growth was noticed at 6 μg/ml. of nystatin and there was complete inhibition of growth above 8 μg/ml. In the case of retarted growth, the hyphae became closely septate, swollen and slightly deformed. Similar observations were made in *Rhizoctonia solani* also at concentrations of 15 μg/ml. of nystatin. *Pythium aphanidermatum* showed no morphological changes or variations in its cultural characters even at the highest concentration of nystatin tested (1000 μg/ml.).

Seed Treatments

(a) *Pythium aphanidermatum* inciting damping off in chillies (*Capsicum annuum*) is an important disease in many parts of the country. Use of filipin in the control of damping off has been reported by Gattani[3] in *Carthamus* sp. Gregory *et al*[4] have used a number of antibiotics for control of damping off incited by pythium species. The results of seed treatment are given in Table I. The seeds were planted in sterilized soil to which pure culture of *Pythium aphanidermatum* grown on corn-meal-sand had been incorporated.

TABLE I.

EFFECT OF SEED TREATMENT WITH NYSTATIN ON DAMPING OFF IN CHILLIES

Dosage of antibiotic for 100 seeds	Seed germination %	Diseased seedlings %
500 mg. ..	80	93
1 g. ..	83	95
1.5 g. ..	81	94
2 g. ..	79.5	96
No treatment ..	84.5	97.5

The results confirm that nystatin has no effect on *Pythium aphanidermatum*.

(b) *Colletotrichum indicum* incites the blighting of cotton seedlings in India

and in some regions becomes epiphytotic. Reddish brown necrosis and cankering of the cotyledons and hypocotyl are general symptoms.

Studies *in vitro* indicated that the pathogen is very sensitive to nystatin and concentrations upto 2.5 μg/ml bring about the disintegration of the germinating spore. The effects of treatment of cotton seeds with nystatin on the disease incidence is given in Table II. The pathogen was, as in previous case, incorporated in sterilized soil before planting the treated seeds.

TABLE II

EFFECTS OF SEED TREATMENT WITH NYSTATIN ON DISEASE INCIDENCE IN COTTON SEEDLINGS DUE TO *C. indicum*
(Average of 5 Replicates)

Antibiotic concentration for 100 seeds	Seed germination %	Diseased seedlings %
30 mg. ..	91	30
40 mg. ..	93	19
50 mg. ..	91	18
No treatment ..	90	98

The data indicate that seed treatment with nystatin gives fairly good protection for the seedlings against infection. However, comparative results of treatments with known chemical fungicides have to be obtained before evaluating the merits of nystatin.

(c) *Macrophomina phaseoli* incites charcoal rot and seedling blight of large number of plants in the country. Moniz, Thirumalachar and Patel[5] worked out a technique for obtaining high percentage of infection of cotton seedlings by *M. phaseoli*. This method was adopted for evaluating the effects of cotton seed treatment with nystatin for protection against *M. phaseoli*. The results are given in Table III.

TABLE III.

EFFECTS OF SEED TREATMENT WITH NYSTATIN ON DISEASE INCIDENCE IN COTTON SEEDLINGS DUE TO *M. phaseoli*
(Average of 5 replicates)

Antibiotic used for 100 seeds	Seed germination %	Diseased seedlings %
100 mg. ..	91	30
150 mg. ..	90	12
200 mg. ..	80	11
No treatment ..	91	95

The data indicate that nystatin in fairly high concentrations protects cotton seedlings from wilting incited by *M. phaseoli*. However, charcoal rot disease may appear in later stages of growth of the plant, since the organism is widespread in soil and attacks the plant in all stages of growth.

(d) *Rhizoctonia solani* incites damping off, blight and various other root diseases of plants. It has a wide host range and damage caused to crop plants is considerable. In the present study, the effect of treatment of tomato seeds with nystatin in protecting the seedlings against *R. solani* was studied. As in previous instances, the inoculum prepared from pure culture growth of the fungus on corn-meal-sand was incorporated into sterile soil and the treated seeds were planted.

TABLE IV.

EFFECT OF TREATING TOMATO SEEDS WITH NYSTATIN ON DISEASED SEEDLINGS DUE TO *Rhizoctonia Solani*
(Average of 5 replicates)

Antibiotic used for 100 seeds	Seed germination %	Diseased seedlings %
250 mg. ..	61	36
300 mg. ..	60	26
350 mg. ..	58	24
No treatment ..	83	90

The data indicate that nystatin treatment has resulted in fewer number of diseased seedlings than the untreated ones. However, there was indication that nystatin may delay seed germination resulting in many cases of pre-emergence damping off. This accounted for the low percentage of germination in the treated lots.

General Remarks

From the above data it may be concluded that nystatin may be useful as seed treatment only in the case of *Colletotrichum indicum* on cotton. The antibiotic is non-phytotoxic and the disease inciting organism is susceptible to low concentrations of nystatin. Hence it may prove more beneficial than the other organic fungicides and mercurials used at present. These data become valuable only when we are able to manufacture this antibiotic in sufficiently large quantities at low cost.

ACKNOWLEDGEMENT

The senior author is grateful to Dr. E. Gonsalves, Professor of Botany, Institute of Science, Bombay, for kind encouragement and facilities for research.

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A Simple Air-Lift Laboratory Bottle Fermentor

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LUNDGREN and Russel[1] recently described a 1 to 5 gal. capacity laboratory bottle fermentor utilizing the principle of air-lift pump for aeration and circulation of the medium. The device was a modification of the Scholler and Seidel's[2] plant-size apparatus in which the circulation tube was within the bottle. Lundgren and Russel's fermentor has certain disadvantages in that the bottle has to be kept inverted and the fermentor end of the air outlet tube being inside the empty space in the bottle there is every chance of the medium being expelled out in case of excessive foaming. Besides, they have used two T-tubes for the primary and secondary air inlets which are also used for inoculation and sampling. On account of the inverted position of the bottle these parts are also liable to leakage due to hydrostatic pressure.

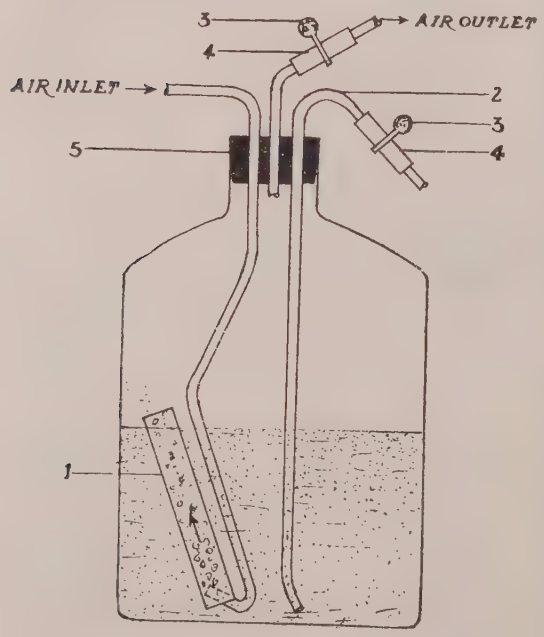

Fig. 1. Air-lift bottle fermentor.
1. Liquid-lift tube. 2. Sampling tube. 3. Pinch clamp.
4. Rubber tubing. 5. Rubber cork No. 12

In order to overcome some of these difficulties the following simple modification has been devised using an upright bottle and a three-holed rubber cork to accomodate one air inlet tube, one air outlet tube and one sampling tube. Fig. 1 shows the functional diagram of a 12-litre Pyrex bottle fermentor. To the end of the air inlet tube the liquid lift tube (Pyrex tube, 160 mm. long, 22 mm. bore) is fused. The stem of the inlet tube is twice bent in such a way that the airlet and liquid-lift tubes can easily be inserted into the bottle as a whole. The bendings should be done at two different planes such that the liquid-lift tube rests against the side of the bottle at an angle as shown in the diagram. The upper end of the lift tube should be slightly above the liquid level so that by forcing air through the inlet tube the liquid medium will be lifted from the bottom and thrown against the bottle wall at the same time effecting a continuous circular movement of the medium as a whole. For taking samples the air outlet is momentarily closed and the pinch cock on the sampling tube released.

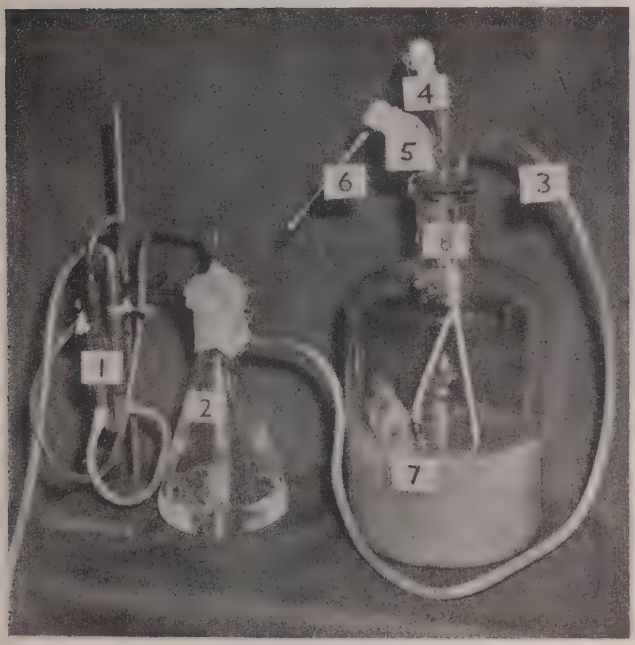

Fig. 2. Set up of air-lift bottle fermentor.

1. Air filters.
2. Humidifier.
3. Air inlet.
4. Air outlet.
5. Additional air outlet (optional).
6. Sampling tube.
7. 12-litre Pyrex bottle.
8. Liquid-lift tube.

With a 12-litre pyrex bottle 6-8 litres of medium could be effectively aerated and agitated for growth of bacteria and actinomycetes ; fungal growth becomes rather too thick for adequate aeration necessary for antibiotic production.

The complete fermentation set up is shown in Fig. 2. Air is sterilized by passing through two metal air filters packed with glasswool and then humidified by passing through sterile water in a filtering flask before entering the fermentor. In the fermentor the tubes are all made of stainless steel. An additional air outlet has been provided but is not essential. The end of the sampling tube has been provided with a long screwcap stopper which can easily be flamed and manipulated while taking samples.

For sterilization it has been found convenient to sterilize the empty bottle separately and then introduce the medium sterilized separately in a number of 2 litre flasks. The air filter, humidifier and the stopper unit are sterilized together as one unit. After adding the medium and inoculum the rubber stopper unit is fitted on the bottle. If the tubes are of stainless steel they could be easily inserted through the bottle neck under flame.

Addition of antifoam or nutrients at later stages can conveniently be made through the air outlet tube.

Experiments are now in progress to study production of actinomycins and other fermentations in the bottle fermentor.

ACKNOWLEDGEMENT

The author is indebted to Dr. S. K. Das Gupta, Mr. G. P. Sen, Mr. R. S. Phadke and Mr. B. N. Ganguli for assistance.

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"Maxipen", A New Synthetic Penicillin*

There are now available several antibiotics effective against diseases amenable to penicillin treatment, but within its own range of activity penicillin is unsurpassed and remains the drug of choice. In addition, of all the drugs ever given, penicillin is perhaps the least toxic, and this remains true despite the fact that a number of people have become allergic to it.

Penicillin G is largely inactivated even in moderately acid solutions, such as is normal in the stomach. Hence, best success is obtained only by injection. Injections, however, require the services of a physician, and may be discomforting to the patient. In recent years, therefore, research has been directed to design an oral penicillin which could resist the acidity of the gastric juices, and hence could pass from the gastro-intestinal tract into the blood stream without significant loss of activity.

Maxipen (Pfizer) is a new oral penicillin produced by coupling 6-amino-penicillanic acid (6-APA) — the nucleus of the penicillin molecule — with ∝-phenoxypropionyl chloride. Production of 6-APA was accomplished by a fermentation process, based on an emzymatic hydrolysis of penicillin G to 6-APA and phenylacetic acid. From screening studies several bacteria possessing the enzyme have been isolated.

The Pfizer method for producing 6-APA is completely different from that reportedly developed by the Beecham-Bristol group. It is understood that the latter make 6-APA by direct fermentation and not by starting with penicillin G. The Pfizer process, on the other hand, involves :

1. Propagation of a selected strain of microorganism through several fermentation stages.
2. Addition of penicillin G to the fermentor after sufficient cell growth has been obtained.
3. Reaction of the penicillin G to form 6-APA.
4. Selective recovery of the 6-APA.

With the nucleus available in quantity, a number of methods of linking the second major building-block of the penicillin molecule — the side chain — were developed. It has been possible to attach many side chains to the molecule by these methods so that more than 1200 new penicillin compounds have been produced. Many of these compounds show unusual and unexpected biological activities, and are now undergoing further study. *Maxipen* is one of the acid-stable, highly absorbed oral penicillins, discovered by Dr. Huang and Dr. Seto after a large number of synthetic penicillins had been made and tested.

The anti-bacterial spectrum of *Maxipen* is substantially the same as that of penicillin G, or of oral penicillin V. However, when taken orally it produces blood levels nearly twice as high as those afforded by penicillin V, and gives higher blood levels even when taken with meals than are attainable with penicillin V, in a fasting stomach.

Chemically *Maxipen* is ∝-phenoxyethyl penicillin, with two optical isomers. Of these, the more active one against bacteria, is the L-isomer. *Maxipen* contains about 60-70 per cent of the L-isomer, in accordance with U.S. Federal analytical standards, which specify that a product of this kind must not contain less than 25 per cent of the D-isomer.

Maxipen does not appear to be active against bacteria resistant to other forms of penicillin, nor does it appear to be less allergenic than other oral penicillins. However, oral penicillin generally produces fewer and less severe allergic reactions than do the parenteral forms of antibiotics.

* Courtesy: Dumex Private Ltd., Bombay 1.

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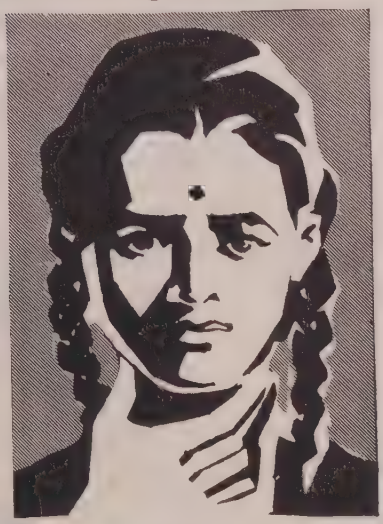

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ISLC-3

5. Glaxo Laboratories Ltd.*

Ulverston, Lancs.

GLAXO Laboratories Ltd. is a Commonwealth organisation with its origins in New Zealand. Nearly a century ago the founder of the group, one Joseph Nathan, arrived in the Antipodes with the early settlers and acquired a trading business that was the forerunner of today's Glaxo world-wide organisation.

The firm of Nathan, supplying goods to the settlers began exporting surplus products to Britain and the enterprise was so successful that in 1826 a London office was set up. In 1904 the Company began importing milk drying equipment into New Zealand with the object of producing milk powder for infant feeding. This signalled the introduction of Glaxo infant food (which incidentally gave the Company its name) and at the same time assured the organisation of a tremendous future.

An important event in the Company's history occurred in 1924 with the manufacture of *Ostelin*, a vitamin D concentrate, for it marked the metamorphosis of the Company from a purely infant food producer to that of a pharmaceutical concern.

It was, however, the production of penicillin towards the end of the Second World War that marked the major turning point in the Company's history. With several other British producers Glaxo was making penicillin by the surface culture method — a laborious procedure, yielding only small supplies, involving culture in glass bottles. Because of the urgent need for the drug, the Company leased a number of south of England factories for penicillin production.

Towards the end of the war Glaxo began operating, in conjunction with the Ministry of Supply, a specially erected penicillin factory at Barnard Castle, in County Durham. Designed originally for surface culture production the factory was later converted to deep fermentation manufacture using 10,000 gallon fermentation tanks. At the end of hostilities the plant was acquired by the Company from the Ministry thus placing the Company in a position to make its entry into the peacetime antibiotics field. The Company and its staff are proud of the fact that over 80 per cent of the British produced penicillin available on the Normandy beachhead on D-Day came from Glaxo.

The world demand for penicillin became so great that in 1948, three years after the Barnard Castle factory had begun operations, the Company built a second antibiotics unit. The new factory 75 miles across the Pennines at Ulverston in North-West Lanchashire incorporated all the known improvements and modifications in antibiotic manufacture.

The erection of the factory was destined to be one of the fastest building operations in the history of the Company. The work commenced at a time when Britain was in the grip of an arctic winter and when there was an acute fuel shortage, yet the project went ahead at an astonishing speed. Sixteen months from the laying of the foundation stone, on 14th April 1948, the first fermenter was seeded and a few days later the first batch of penicillin was produced. The remaining fermenters were installed at the rate of one every two weeks. The plant has been built in fairly open country a few miles from the town of Ulverston and is a prominent landmark. The impressive multi-storey production and laboratories block, with its clean lines and distinctive centre tower is of typically modern design. In 1944 when Glaxo decided to manufacture strepto-

* Courtesy: Glaxo Laboratories (India) Private Ltd., Bombay.

mycin, the new Ulverston factory was able to go into full production with this drug at the same time keeping up with the requirements for penicillin.

Today Ulverston has grown to become the Company's major antibiotics production unit with Barnard Castle occupying an interesting role as an ancillary producer plus acting as a "test bed" for new antibiotics requiring large-scale fermentation trials before clinical trials. Besides penicillin and streptomycin other antibiotics manufactured at Glaxo's fermentation plants include neomycin, novobiocin and griseofulvin. The fermentation plant is also used in the production of vitamin B_{12}, *Intradex* (the blood volume restorer) and *Myl-X*, a fungal amylase concentrate used for making bread lighter and softer.

In 1950 the Company set up a Fermentation Research Division at Sefton Park where a full-scale antibiotic screening and development programme is in operation. For ten years soil screening tests in the search of new antibiotics have been carried out (with, in an unpredictable field, some degree of success) and first-rate results have been obtained in producing higher-yielding strains of the micro-organisms employed in penicillin and streptomycin production. Strains currently used are producing twenty times as much penicillin and streptomycin as the original strains and the fermentation vessels are today producing twenty times as much material as previously and virtually without additional working costs.

Sefton Park research staff have figured prominently in recent research on the anti-fungal antibiotic, griseofulvin. In 1955 workers at Sefton Park, with others, recognised that griseofulvin, then being developed by the Company for use in horticulture, was the least toxic anti-fungal antibiotic ever known. Investigations at once began on its possibilities as a therapeutic agent and further work undertaken at Glasgow University confirmed its potential.

While clinical trials were in progress in Austria, Britain and the U.S.A., a team at Sefton Park were busy evolving a deep fermentation method of making griseofulvin which, up to that time, had been available only in very small quantities. The success of this research effort has since been reflected in the tremendous usage, particularly in India, Asia, the Far East and the Americas, of griseofulvin for the treatment of serious fungoid infection.

Glaxo research and antibiotics production staff at Sefton Park and Barnard Castle are at present actively concerned with development work on cephalosporin-C, one of a new group of antibiotics closely related to penicillin but in certain significant respects different both in regard to chemical structure and biological properties. In particular cephalosporin-C is resistant to destruction by the enzyme penicillinase which inactivates penicillin. The work is being directed by the National Research Development Corporation in association with Oxford University Medical Research Council scientists at the Sir William Dunn School of Pathology under Sir Howard Florey and Dr. E. P. Abraham, the Medical Research Council's Antibiotics Research Centre at Clevedon and Glaxo research staff.

Other work, outside antibiotics, has brought the research work by Glaxo Laboratories into prominence during the last 10-20 years. In 1946, for example, an intensive research effort resulted in the successful development of a single preparation for simultaneous immunisation against diphtheria and whooping cough. Work on the production of the whooping cough vaccine itself has featured prominently in the Company's research programme for a number of years.

Ten years later in 1956 a new building was erected at Greenford at a cost of £ 400,000, to be devoted exclusively to biological research and principally to intensify Glaxo work on immunologicals. In it, with the advantages of up-to-date equipment and specially designed laboratories, individual teams work on aerobic

and anaerobic vaccines, histopathology and microbiology. A virus research unit studies virus cultivation and immunity, and an entire wing is given for producing Glaxo freeze-dried BCG tuberculosis vaccine. In 1956, an "Asian" flu' vaccine was ready for the winter epidemic of that year.

Another landmark in the Company's history occurred in May 1948 when E. Lester Smith and his team at Greenford, after some 10 years research, isolated the anti-pernicious anaemia factor from liver (vitamin B_{12}). This vitamin is now manufactured by deep fermentation at Barnard Castle.

Glaxo Laboratories has played an important part in developing the synthesis and production of cortisone. Working in conjunction with the National Research Development Corporation and the Medical Research Council, the Company pioneered the use of hecogenin, a by-product of the sisal industry, as the starting material in cortisone manufacture. Crude hecogenin made in East Africa from sisal is transported to the fermentation plant at the Ulverston factory for purification and later transferred as hecogenic acetate to the Company's cortisone and hydrocortisone plant at Montrose, in Scotland.

Glaxo laboratories has become internationally known for its work on the modified Salk poliomyelitis vaccine. Laboratories for this work were built in the grounds of the Company's Fermentation Research Division at Sefton Park, Stoke Poges, and a steady supply of vaccine is now produced for U.K., and overseas markets.

The Company spends more than a million pounds annually on research and development. More than 250 research staff — about one third of them graduates — work in the Company's Research Divisions at Sefton Park and at the headquarters at Greenford where all other research projects other than antibiotics are handled.

Recently the Company enlarged the scope of its activities by setting up a veterinary department, by entering the animal nutrition field, and by acquiring a synthetic insecticides plant.

The Company continues to make progress in a number of diversified fields. In the last two years Allen and Hanburys Ltd., have merged with Glaxo ; a new food *Amama* has been evolved by Greenford research staff to combat protein deficiency; and new Glaxo factories have been built in Ceylon and Malaya. Of particular interest to India is that the Company's Fine Chemical Division in Bombay is making cortisone and its derivatives.

With headquarters at Greenford in the United Kingdom, Glaxo has subsidiary companies operating throughout the world. The largest outside the U.K. is in India, with offices and factories in Bombay and branches in Calcutta, Madras and New Delhi, in all employing over 2,000 people. Besides administrative offices, the Bombay headquarters at Worli consist of two entirely separate factories, one of them for the primary manufacture of corticosteroids and vitamin A, the other for secondary production where well over one hundred high quality drugs and pharmaceutical products are made. These include immunologicals, vitamin preparations and food supplements. Here also, antibiotics are blended and filled and infant foods packed.

Future expansion plans include the complete processing of infant foods at a factory now under construction at Aligarh, in Uttar Pradesh, and the transfer of the Fine Chemicals factory to a site at Thana. Both these projects are expected to be in operation by the end of this year. The Worli site will then be available, if required, for the development of secondary production, research and other activities.

Other large units among the total of 21 subsidiary companies are in Australia, Argentina, Brazil, Italy, Malaya, New Zealand, Pakistan and South Africa.

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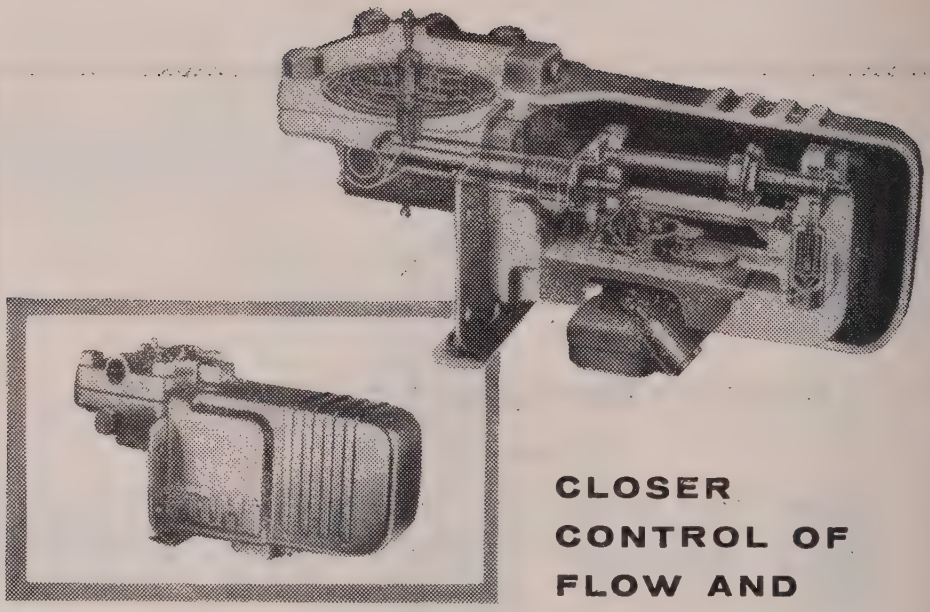

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Edited by Dr. M. J. Thirumalachar and Published by Shantikumar T. Raja, B.A., LL.B. Bar-at-Law, Hindustan Antibiotics Ltd., Pimpri (near Poona). Printed by Rev. Theodore A. Pereira at the Examiner Press, Bombay—1.